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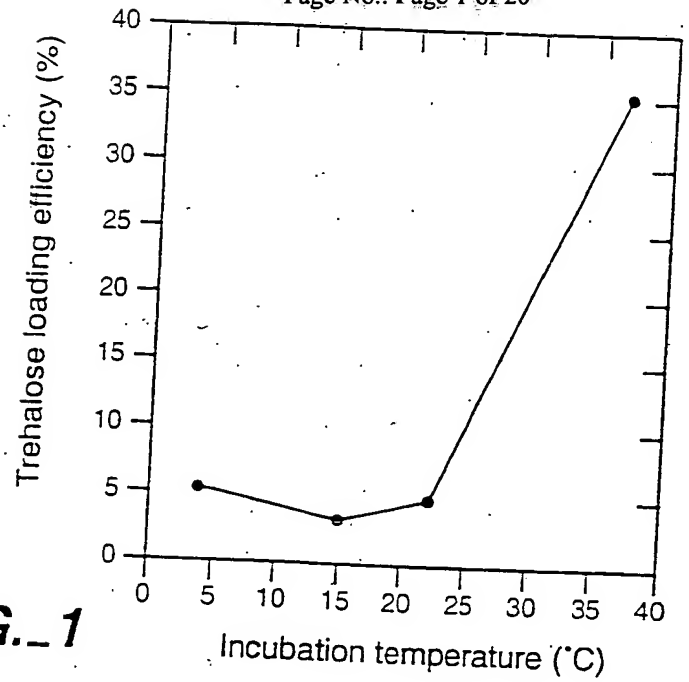

Trehalose loading efficiency (%)
0
5
10
15
20
25
30
35
40
0
5
10
15
20
25
30
35
40
Incubation temperature (°C)

FIG._1

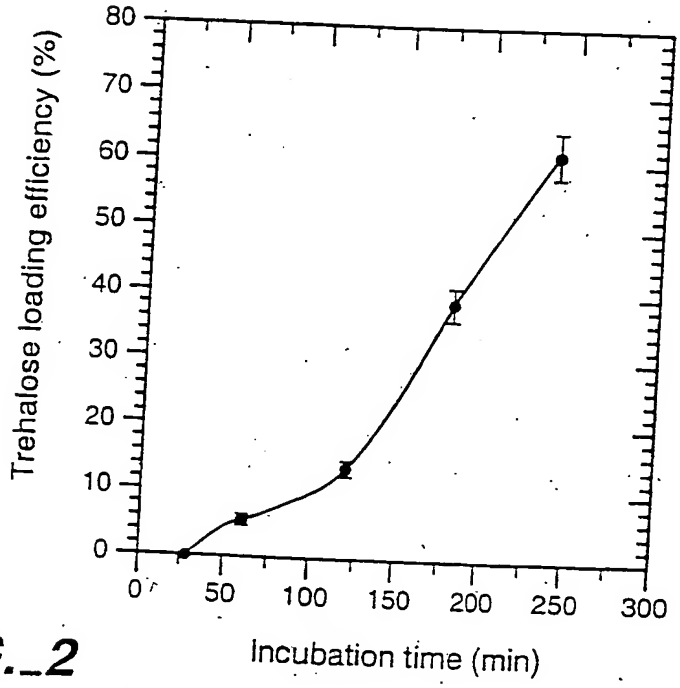

Trehalose loading efficiency (%)
0
10
20
30
40
50
60
70
80
0
50
100
150
200
250
300
Incubation time (min)

FIG._2

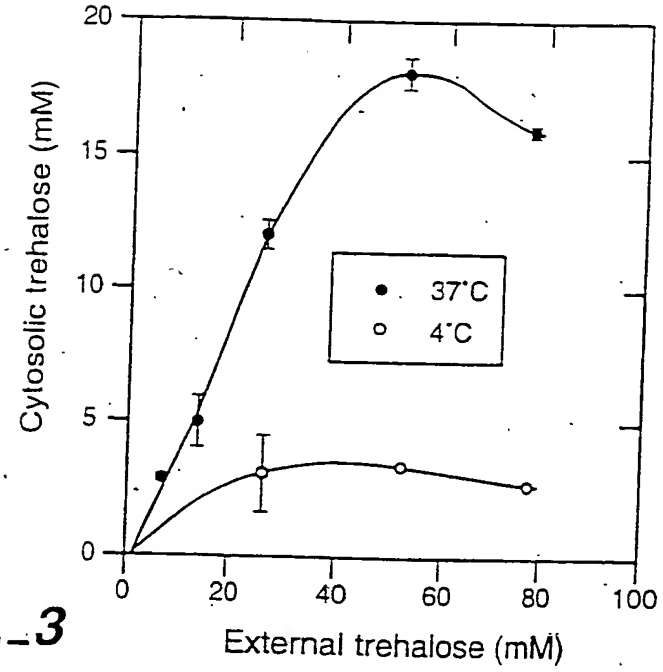

Cytosolic trehalose (mM)
External trehalose (mM)
37°C
4°C
0
5
10
15
20
0
20
40
60
80
100

FIG._3

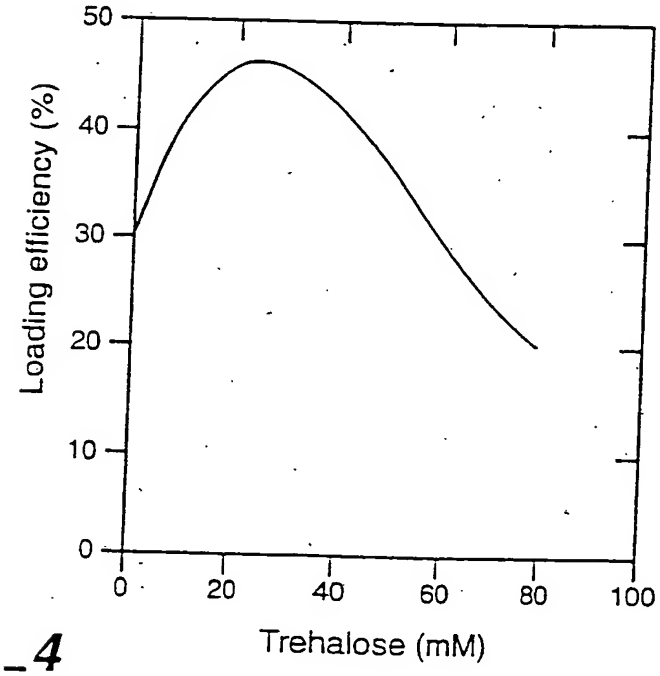

Loading efficiency (%)
Trehalose (mM)
0
10
20
30
40
50
0
20
40
60
80
100

FIG._4

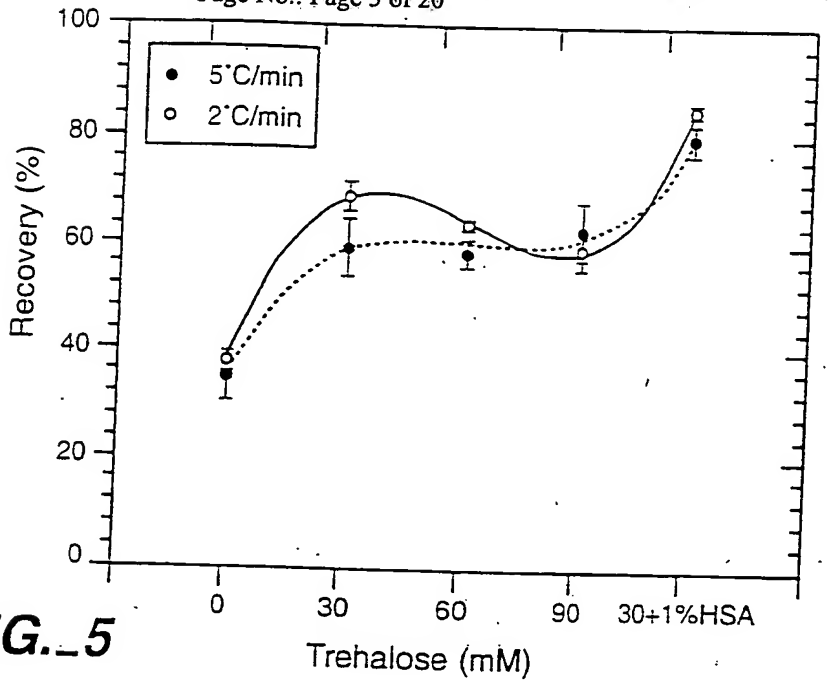

100
80
60
40
20
0
Recovery (%)
5°C/min
2°C/min
0
30
60
90
30+1%HSA
Trehalose (mM)

FIG._5

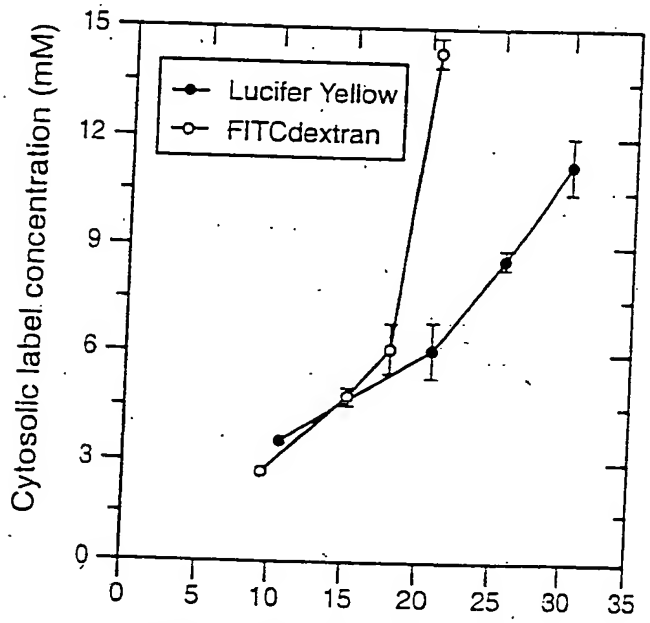

15
12
9
6
3
0
Cytosolic label concentration (mM)
Lucifer Yellow
FITCdextran
0
5
10
15
20
25
30
35
Label concentration in loading buffer (mM)

FIG._6

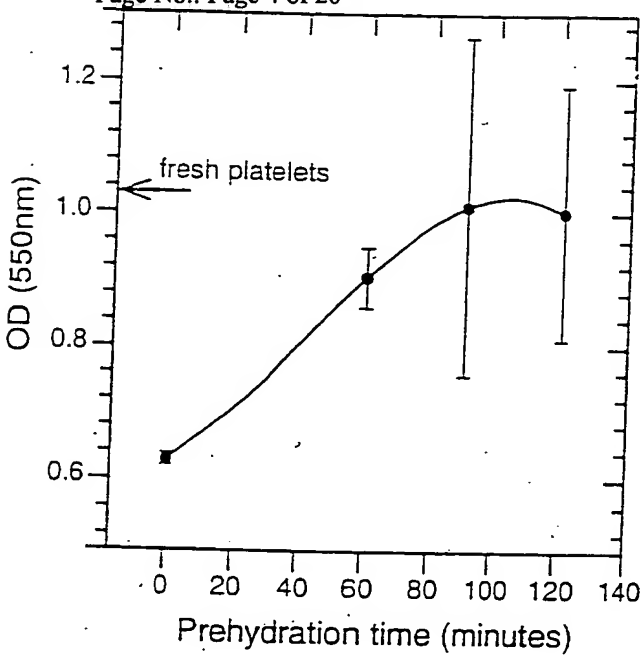


FIG._7

FIG._8A
(PRIOR ART)

FIG._8B

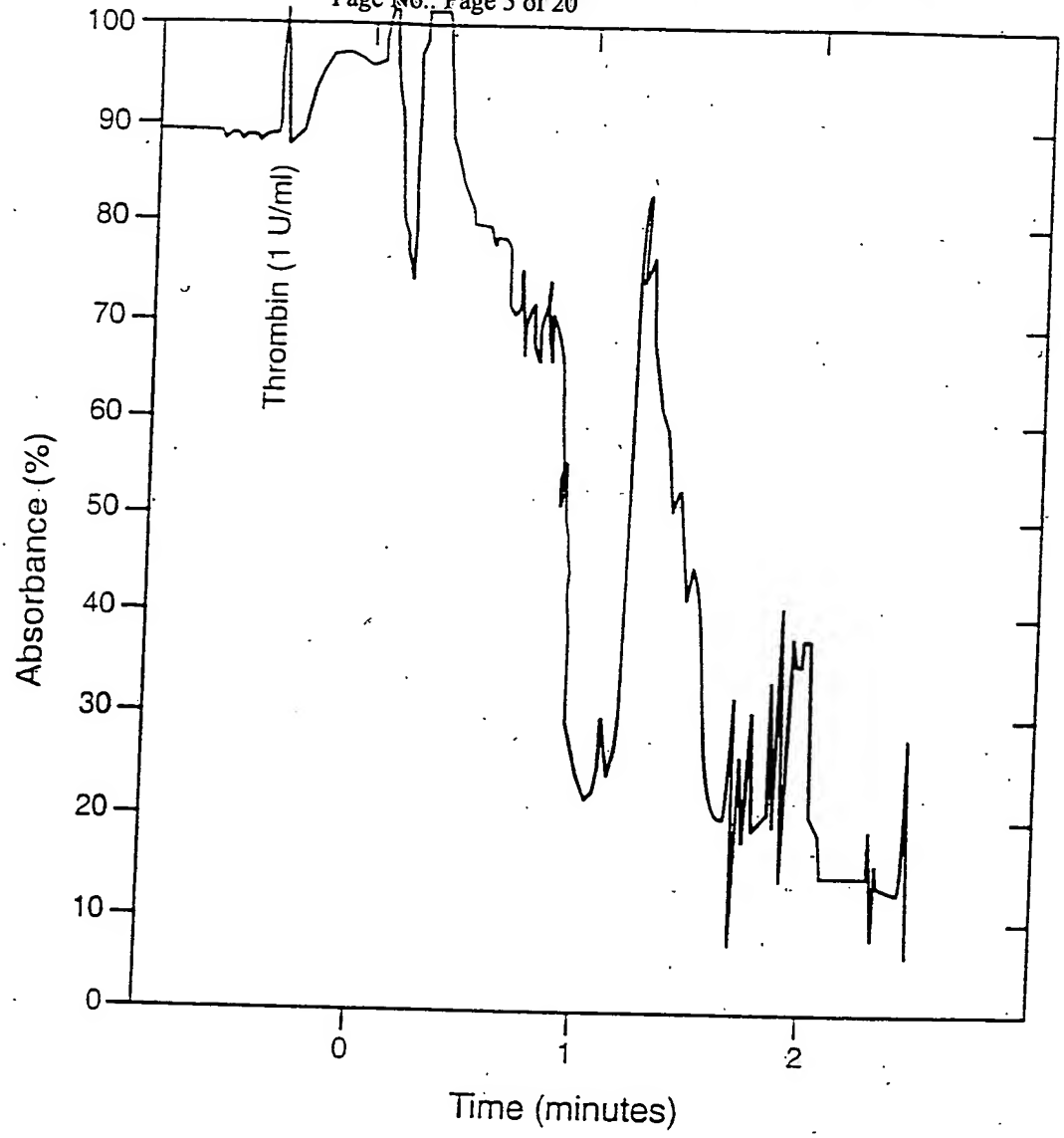

100
90
80
70
60
50
40
30
20
10
0
Absorbance (%)
Thrombin (1 U/ml)
0
1
2
Time (minutes)

FIG._9

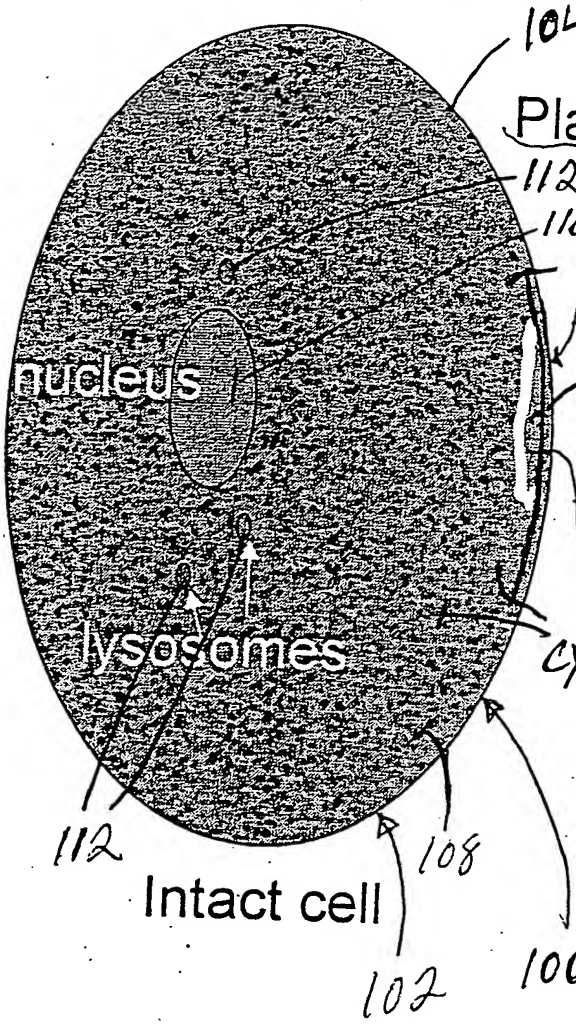


Figure 10

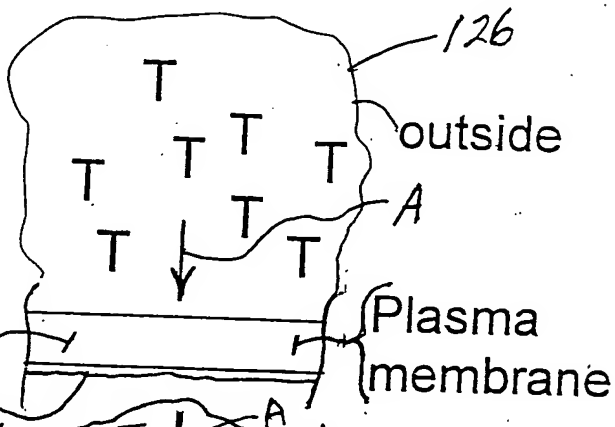


Figure 11

Figure 12

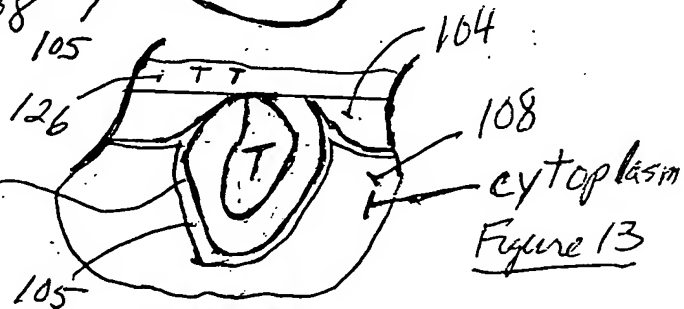


Figure 13

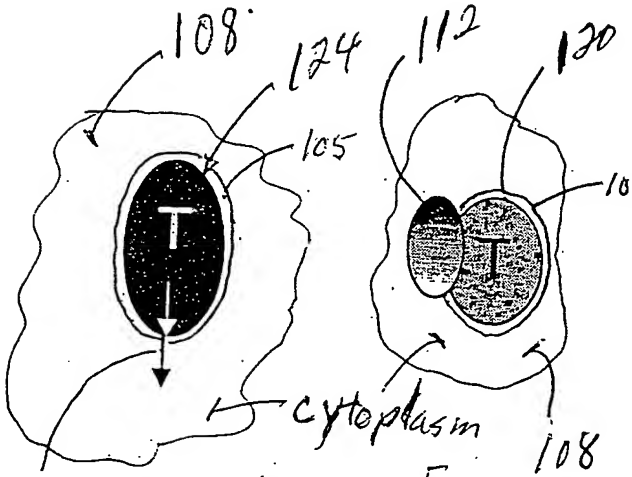


Figure 16

Figure 15

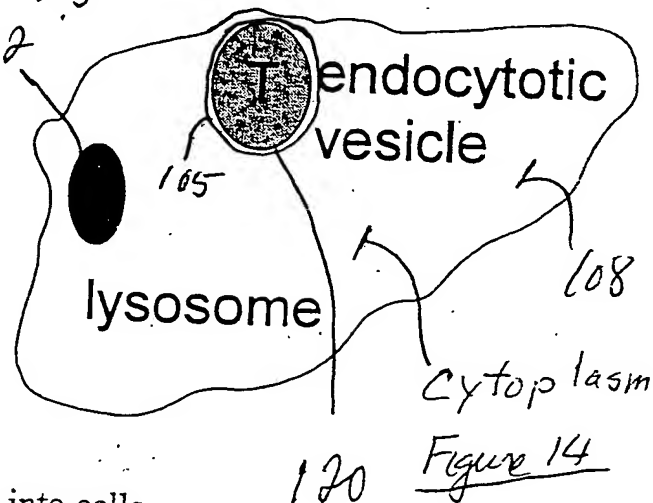


Figure 14

mechanism for loading trehalose into cells.

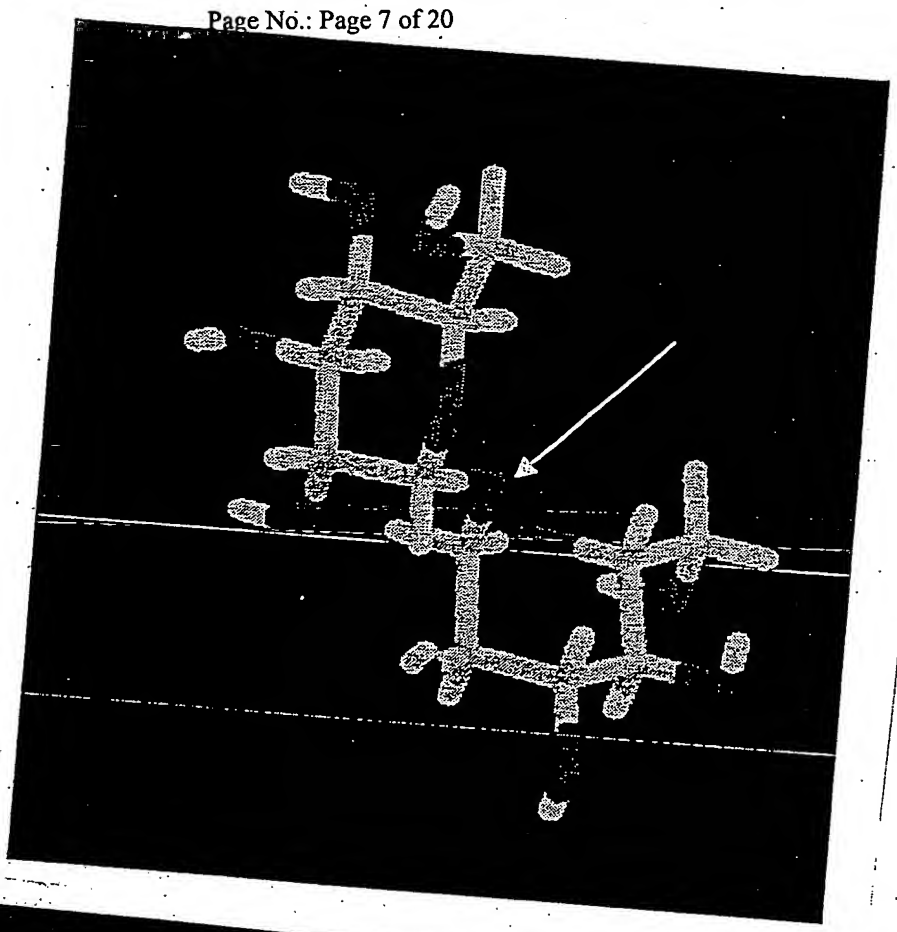


Fig 17

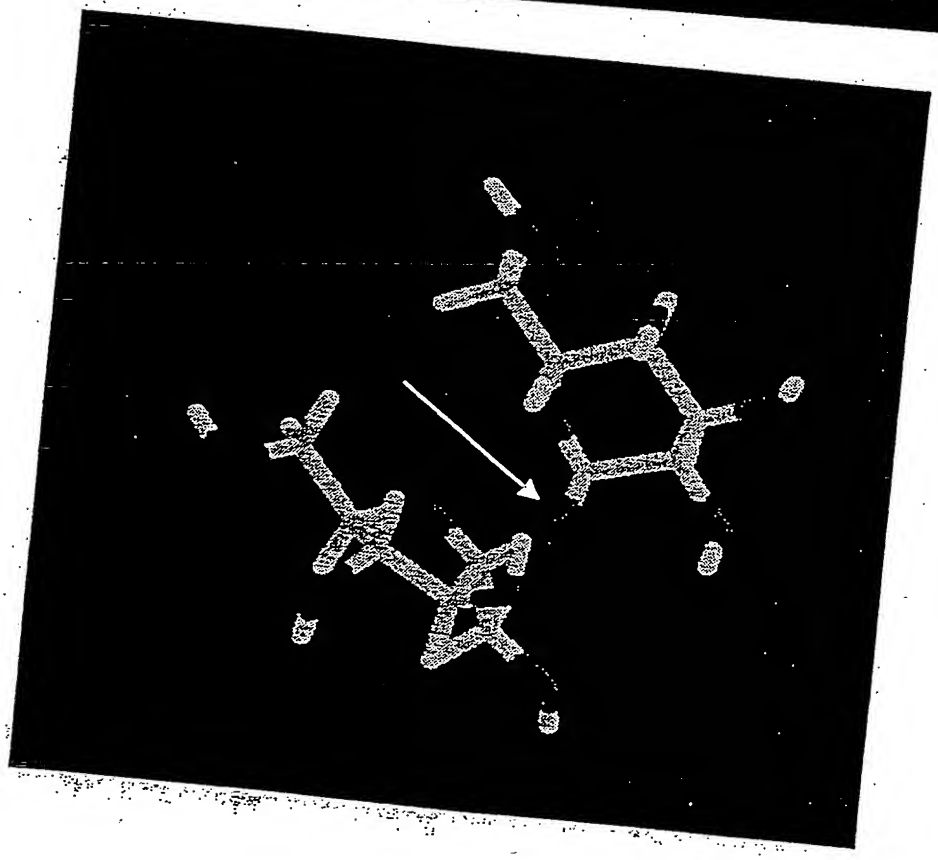


Figure 18

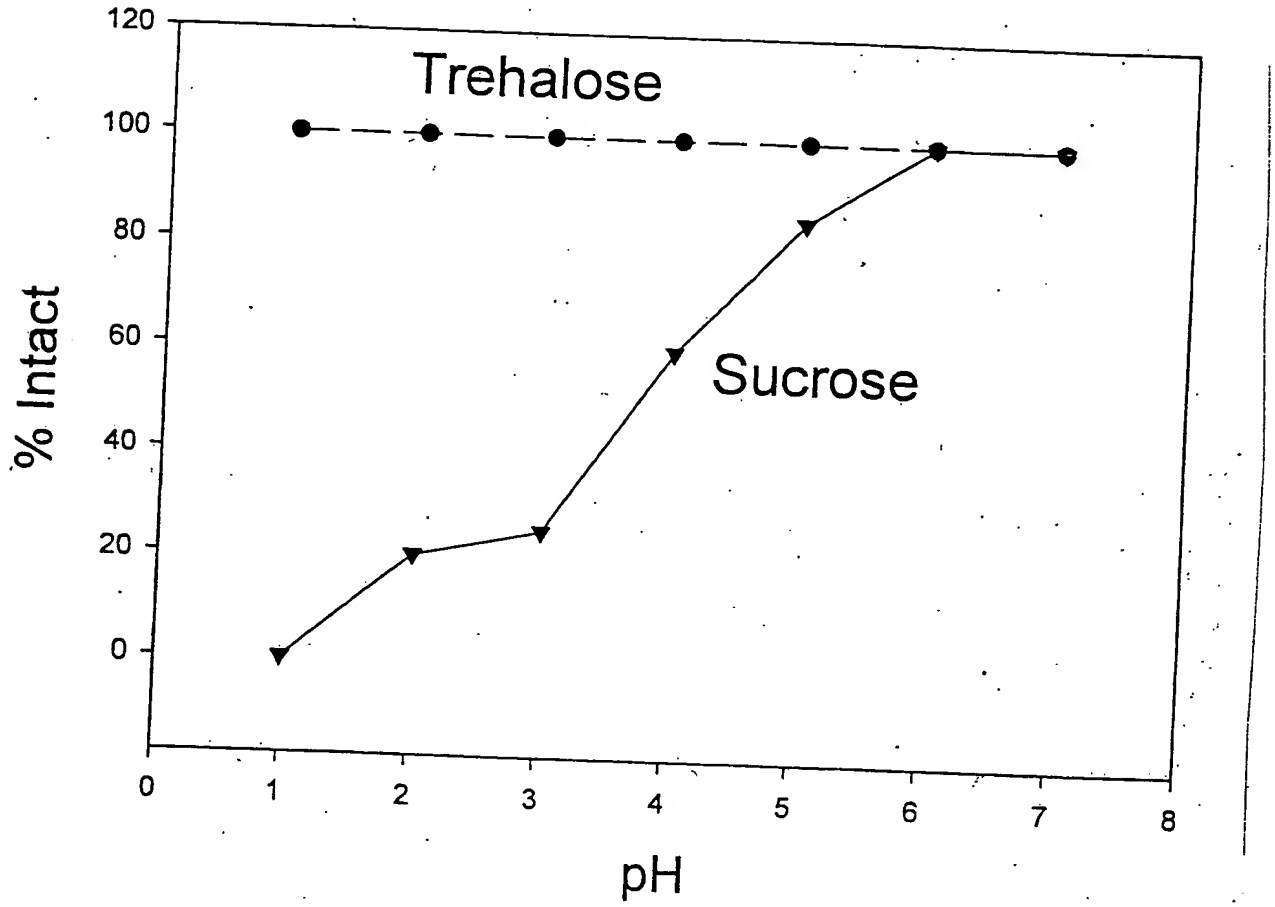


Figure 19

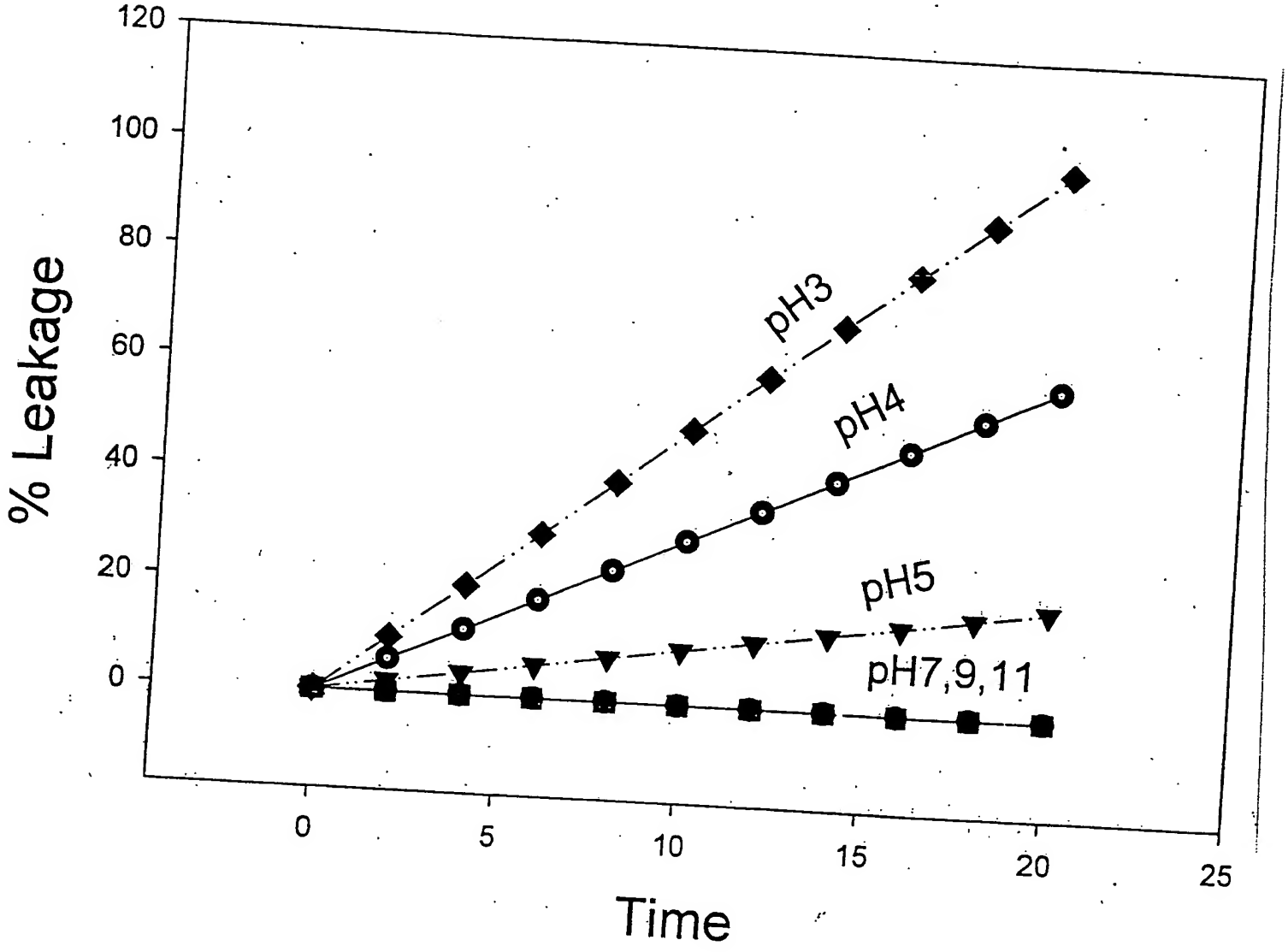

120
100
80
60
40
20
0
% Leakage
pH3
pH4
pH5
pH7,9,11
0
5
10
15
20
25
Time

Figure 20

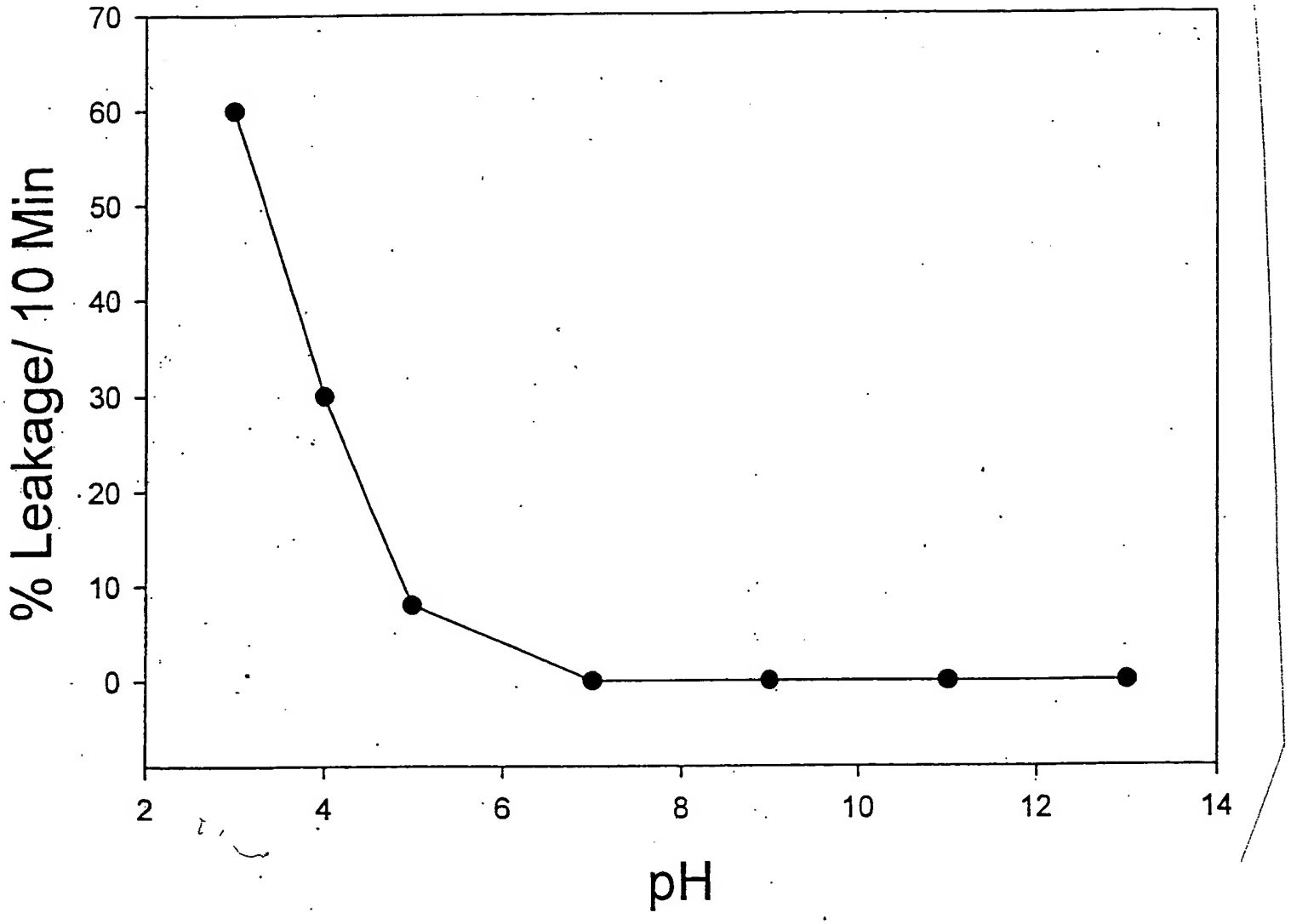


Figure 21

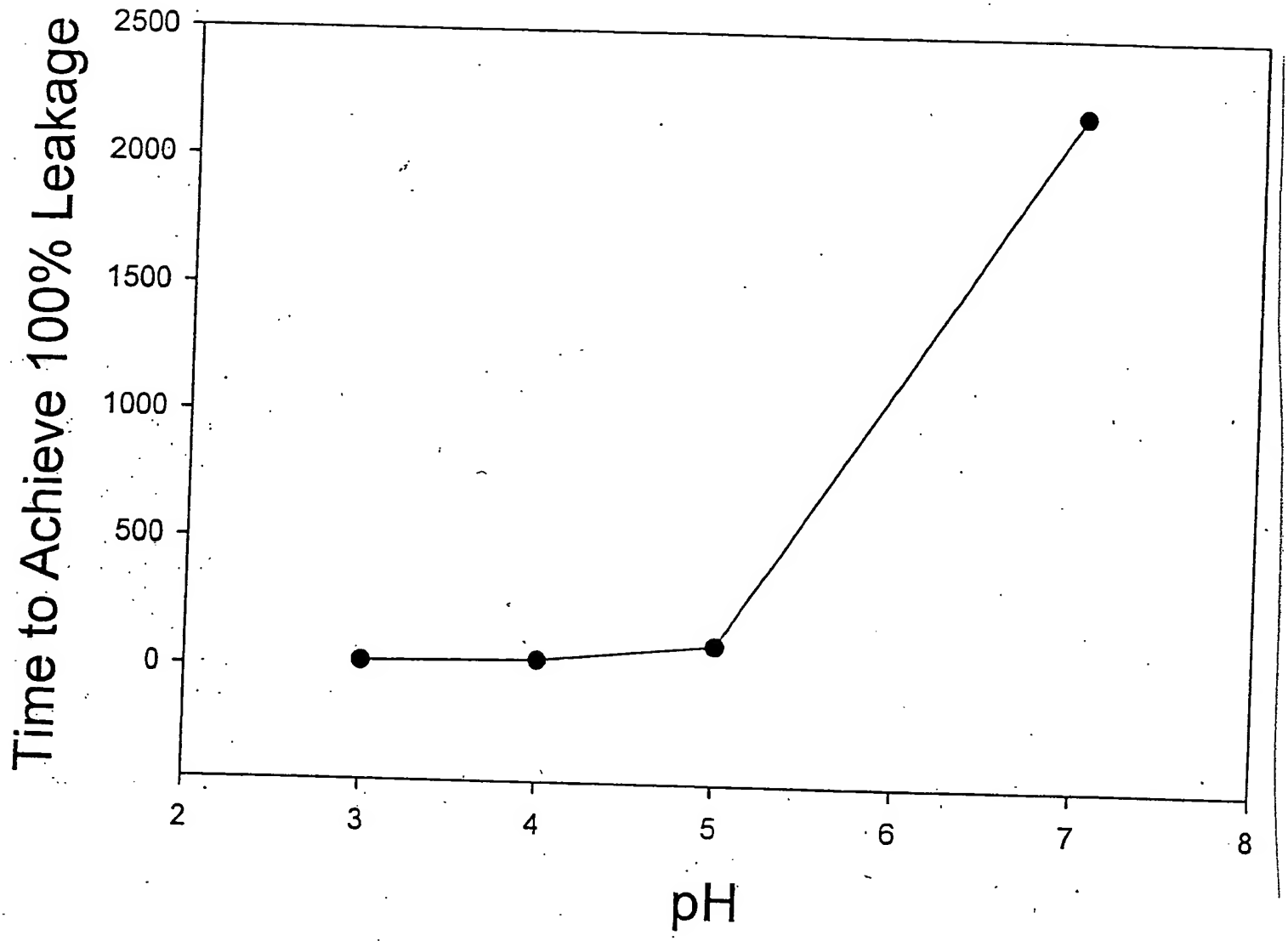
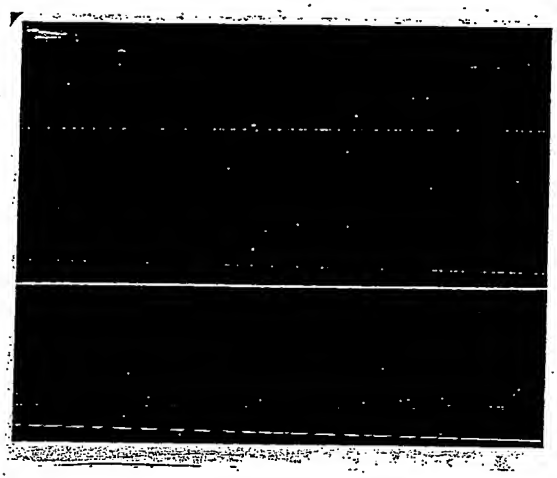
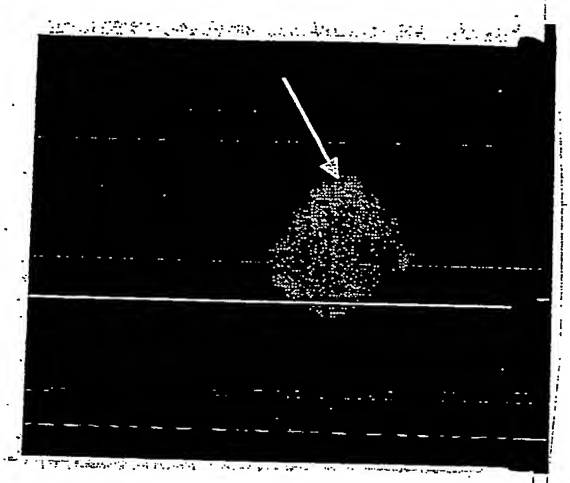

Time to Achieve 100% Leakage
2500
2000
1500
1000
500
0
2
3
4
5
6
7
8
pH

Figure 12



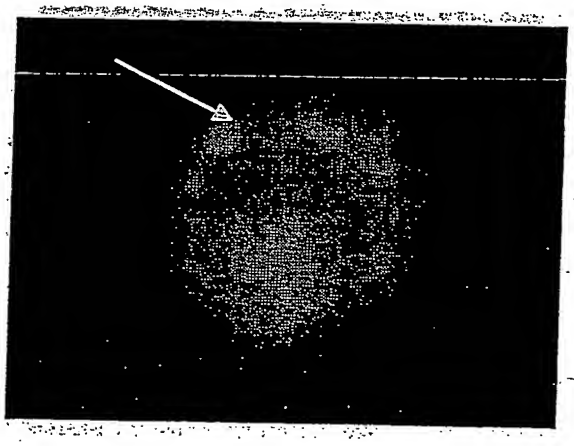
0 hrs (control)

Figure 23



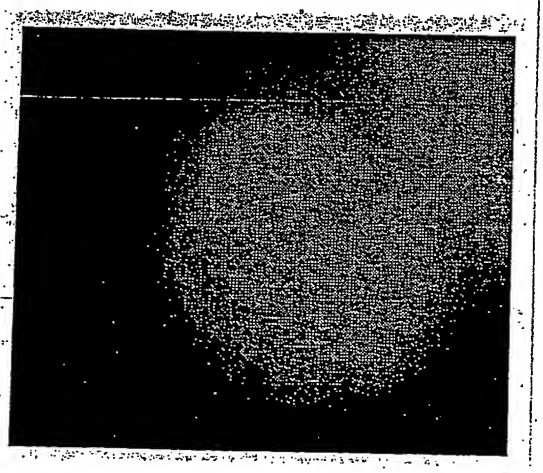
1 hr

Figure 24



3.5 hrs

Figure 25



5 hrs

Figure 26

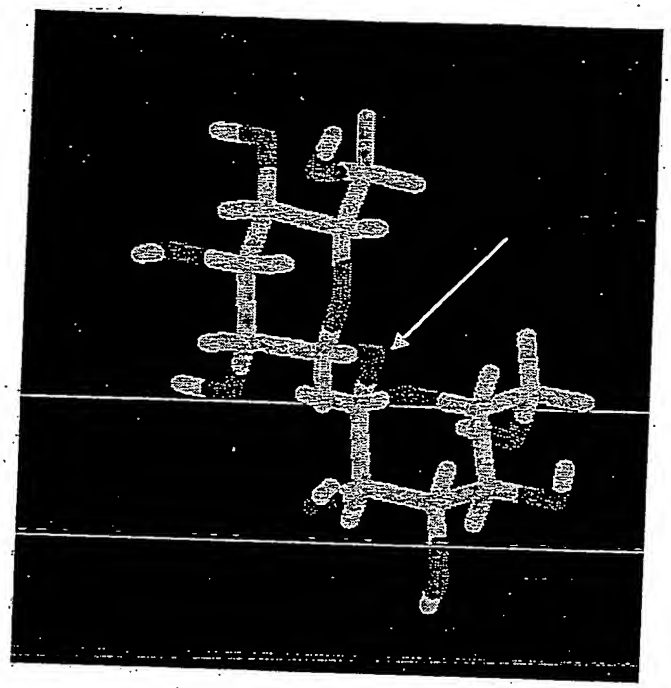


Figure 17

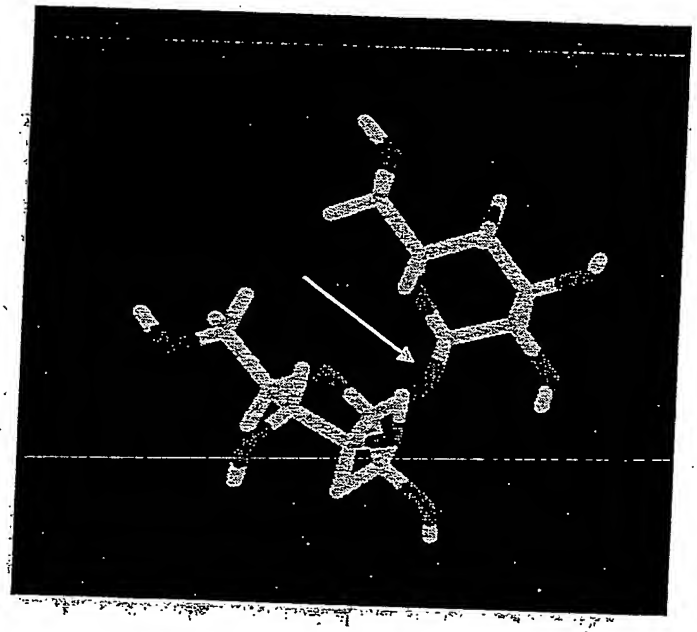
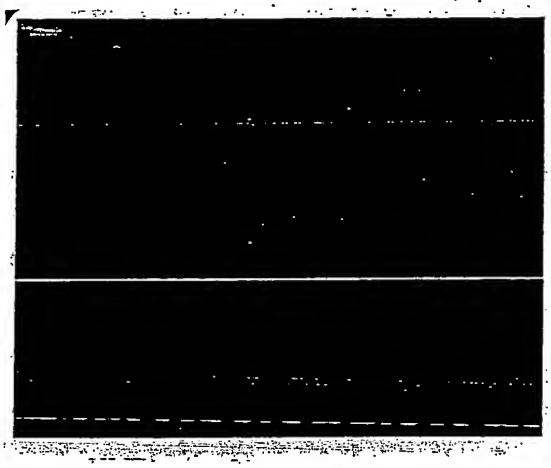


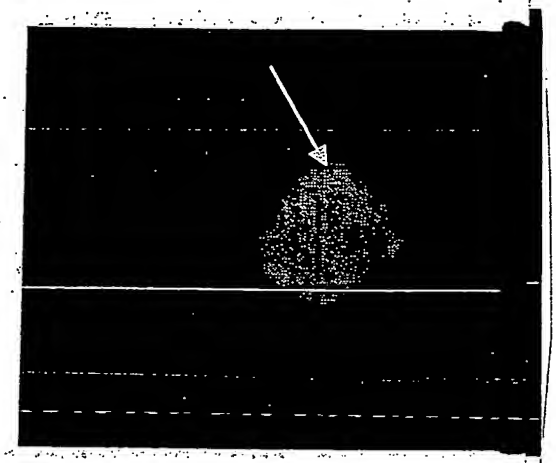
Figure 18

Fig. 2. Trehalose (top) and sucrose (bottom). Trehalose is the only non-reducing disaccharide of glucose. Sucrose is a non-reducing disaccharide of glucose and fructose. The glycosidic bonds, which are known to be susceptible to hydrolysis in sucrose (much less so in trehalose) are indicated by the arrows.



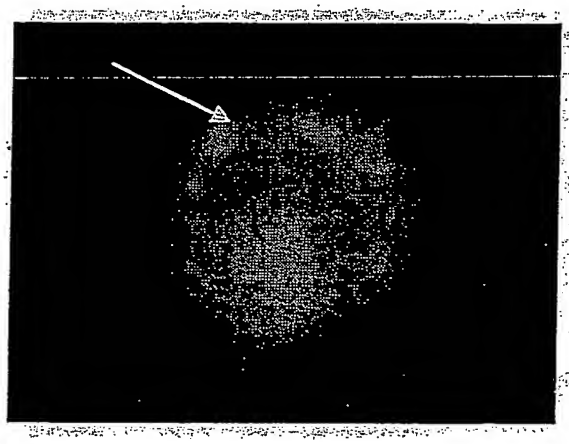
0 hrs (control)

Figure 23



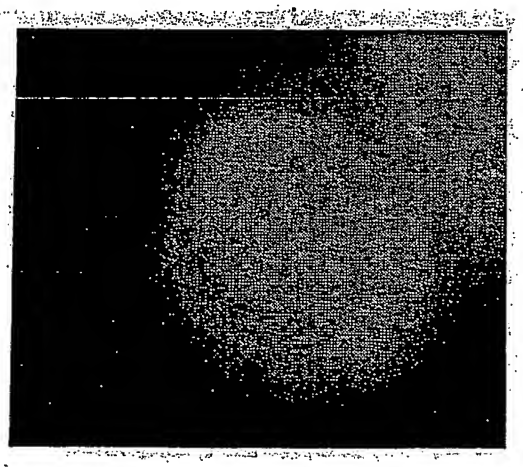
1 hr

Figure 24



3.5 hrs

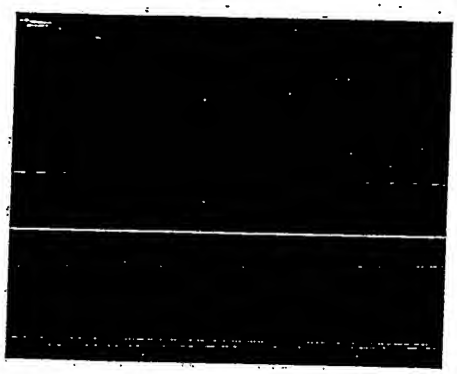
Figure 25



5 hrs

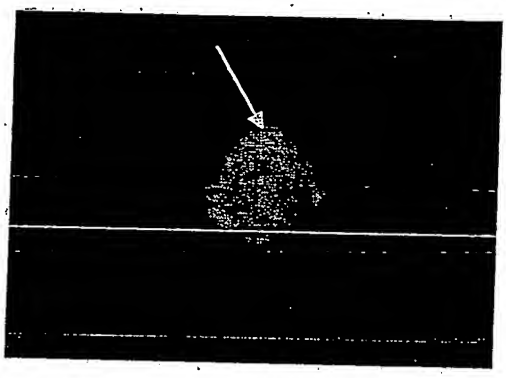
Figure 26

particularly given that the in vitro measurements were done with an artificial system loaded with a large gradient across the membrane. The intact cells, by contrast, have a much smaller gradient across the membrane, and the composition of the biological membrane is clearly quite different from that of the liposomes.



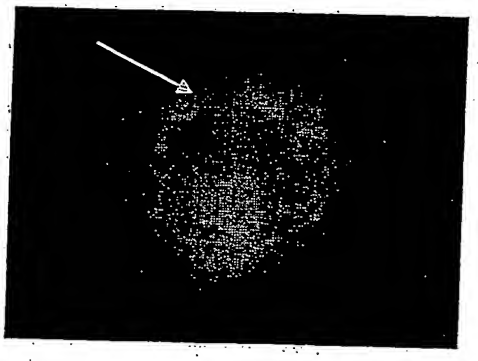
0 hrs (control)

Fig 23



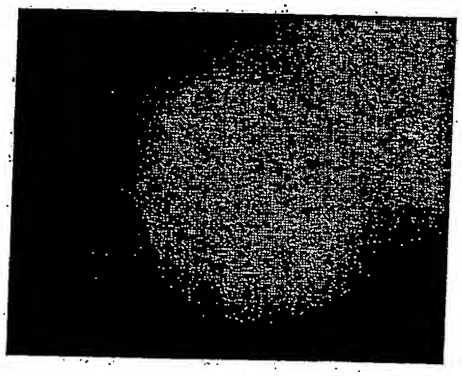
1 hr

Fig 24



3.5 hrs

Figure 25



5 hrs

Figure 26

Fig. 7. Distribution of Lucifer yellow in intact cells as a function of incubation time. At short incubation times the dye is in punctuate structures, presumably endocytotic vesicles. With long incubation times (5 hrs) the staining becomes uniform, suggesting that the dye has leaked into the cytoplasm.

Fig 27

Figure Mesenchymal stem cells were loaded with trehalose for 24 h by incubation at 37 °C in medium + 100 mM trehalose. The cells were either lyophilized in Eppendorf tubes on a Virtis side-arm lyophilizer or air-dried (0.5 mL samples in 35 mm Petri dishes) in a sterile hood to various water contents, They were then rehydrated and viability assessed by trypan blue exclusion. It is clear that, below the critical water content of 2 g H_2O/ g dry weight, the MSCs survived air-drying better than freeze-drying. For some cell types, air-drying might represent the optimal method of drying.

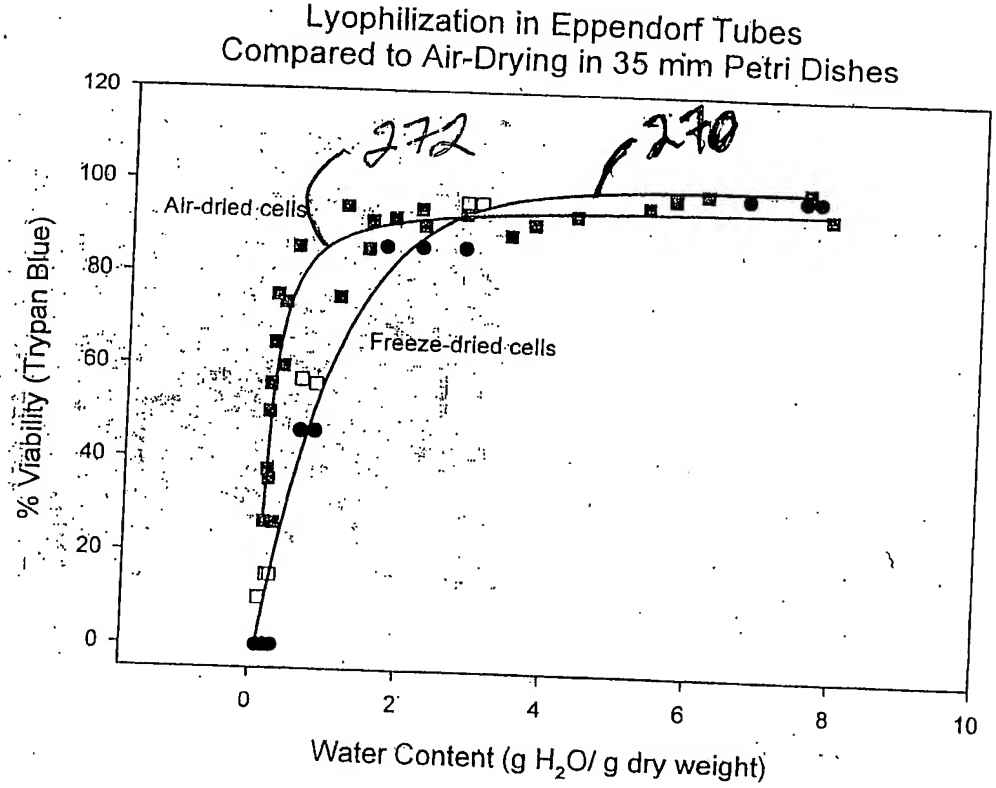


Figure 2. As DMSO is known to cause an increase in membrane permeability, we have addressed the hypothesis that DMSO might improve intracellular distribution of solutes taken up from the extracellular milieu. MSCs were incubated with 10 mM LYCH for 5 h in the presence or absence of DMSO, washed and examined by fluorescence microscopy. In the control sample (Fig. 1A), in which no DMSO was present, the LYCH fluorescence was seen predominantly within endosomes, as indicated by the punctate staining. When 2% DMSO was included for the last 30 min of the incubation, a slightly more diffuse staining was seen (Fig. 1B). The most dramatic result, however, was seen when 2% DMSO was included with the LYCH for the entire 5-h incubation (Fig. 1C). In this case, although some punctate staining was still visible, diffuse LYCH staining was seen throughout the cytoplasm. This result indicates that DMSO may provide some benefit to the cells by aiding in the release of solutes from the endosomes and allowing a more homogeneous intracellular distribution.

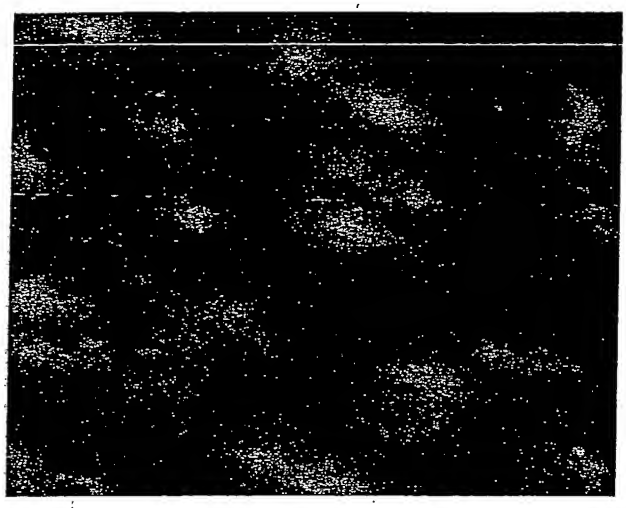


Fig. Control:
LYCH for 5 h; No DMSO

Fig. 28

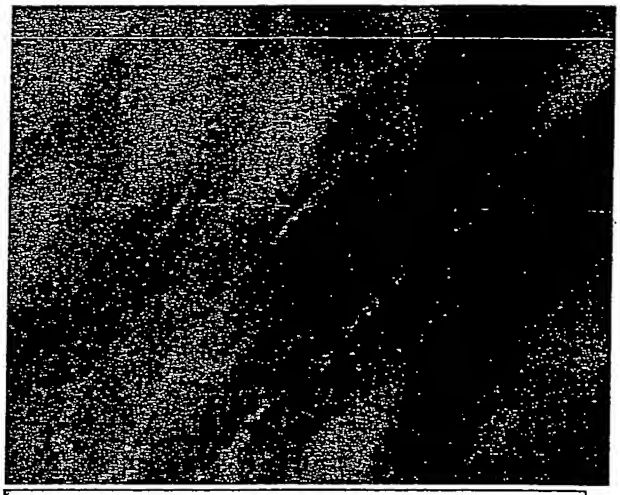


Fig. LYCH for 5 h;
DMSO for final 30 min

Fig 29

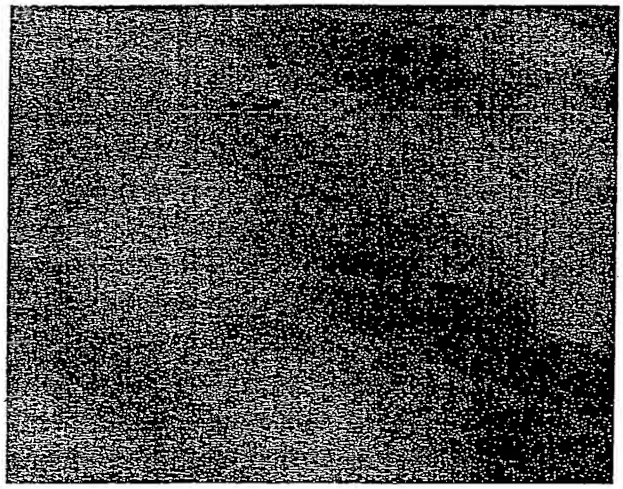


Fig. Continuous Loading:
5 h LYCH & DMSO

Fig 30

Figure DMSO improves the intracellular distribution of trehalose when included with the cells for the full 24-hour trehalose incubation. Mesenchymal stem cells were loaded with 100 mM trehalose for 24 hours at 37 °C. DMSO (2%) was included in the incubation for the full 24 hours, for the last 2 h, for the last 4 h, or not at all (control). The cells were fractionated by differential centrifugation and separated into a nuclear fraction (which also includes unbroken cells:N), a mitochondrial fraction (M), and a lysosomal fraction (L). It can be seen that when DMSO is included in the full 24-hour incubation with trehalose (red bars), the mitochondrial and lysosomal fractions show increased trehalose concentrations as compared to the nuclear fraction, containing whole cells. Treating the samples with DMSO for just the last 2 or 4 hours of the trehalose incubation did not significantly change the trehalose concentrations of the M or L fractions compared to those of the control.

Fig 3.1

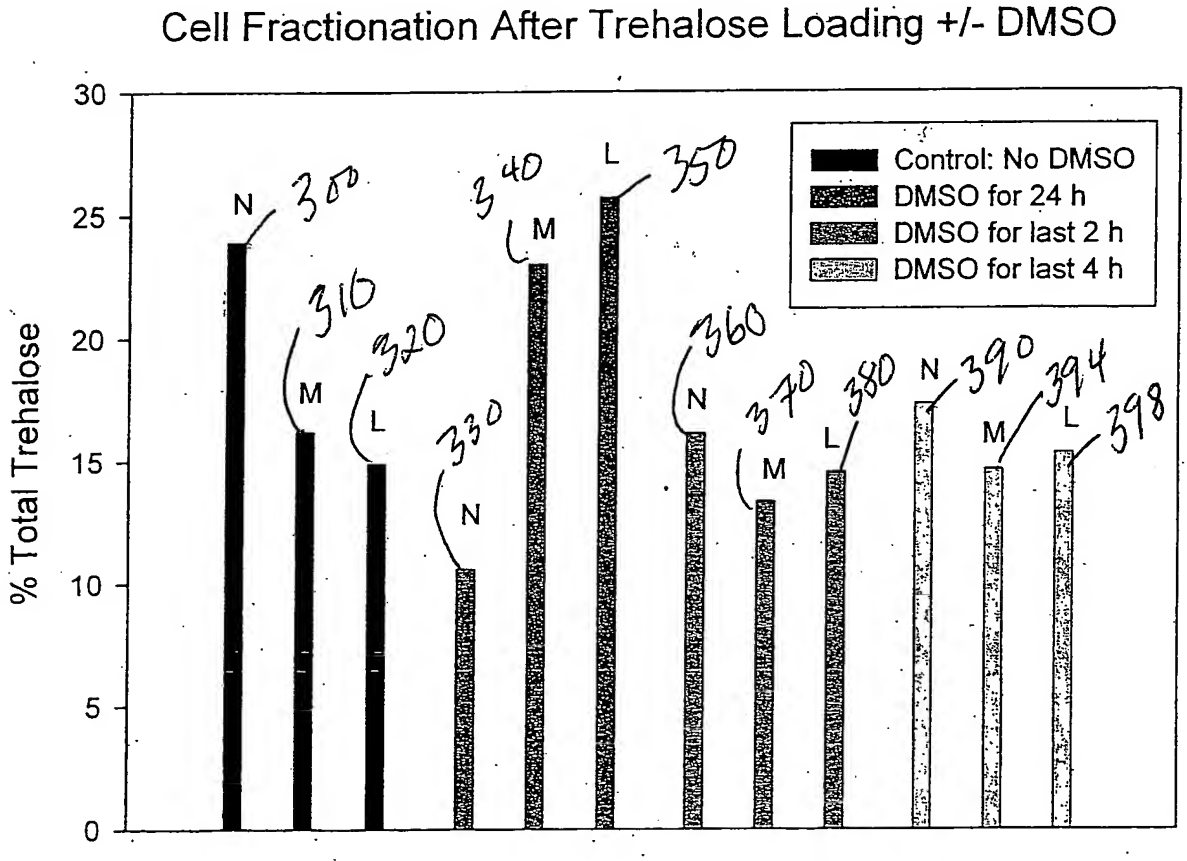
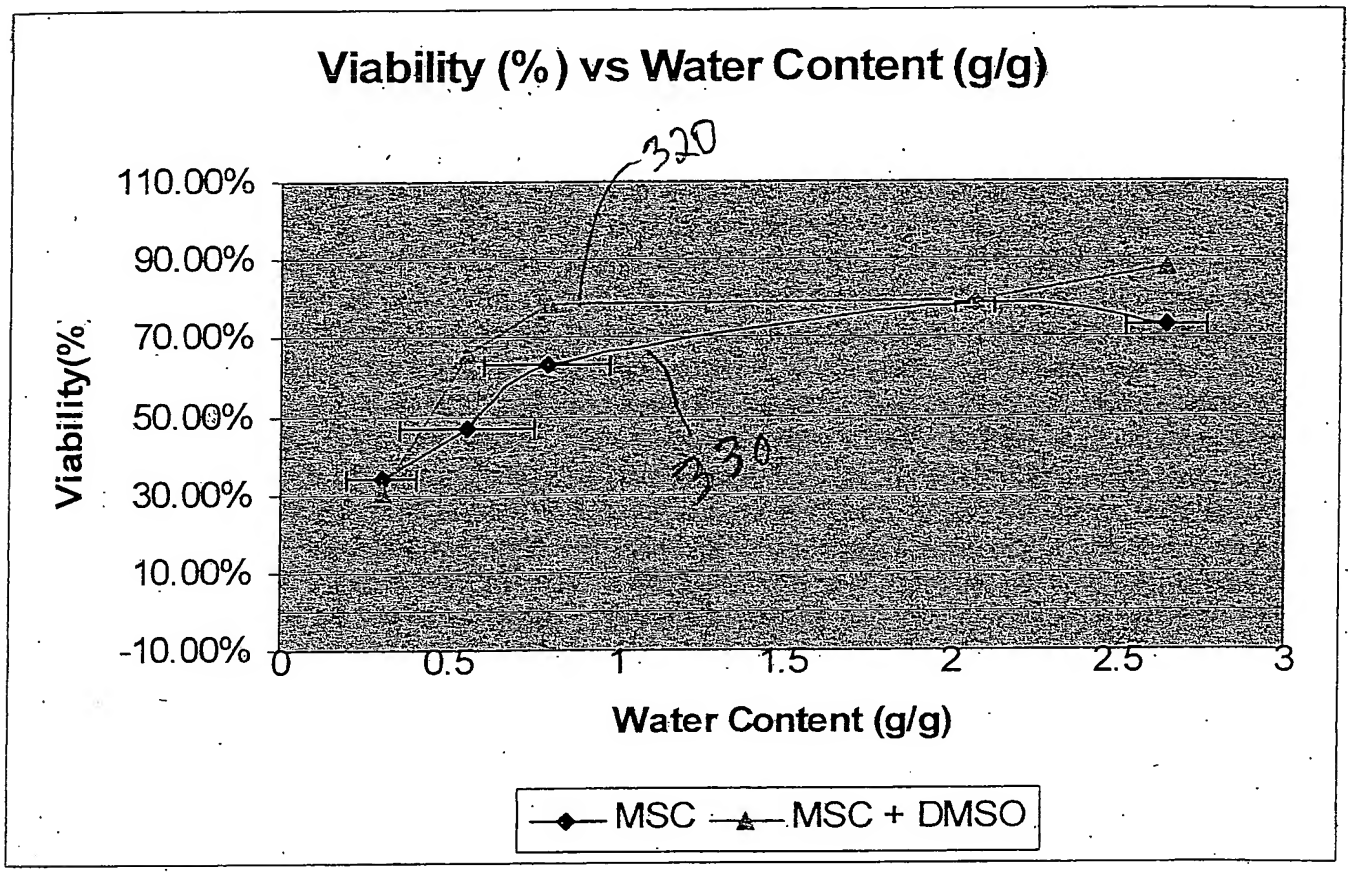


Figure In this experiment, DMSO was shown to aid the recovery of MSCs following air-drying and rehydration. All the MSCs were loaded with 100 mM trehalose for 24 hours. The experimental samples were also treated with 2% DMSO for the last three hours of the incubation. The dried samples were rehydrated with excess medium, and viability was assessed by trypan blue exclusion.

Fig 32



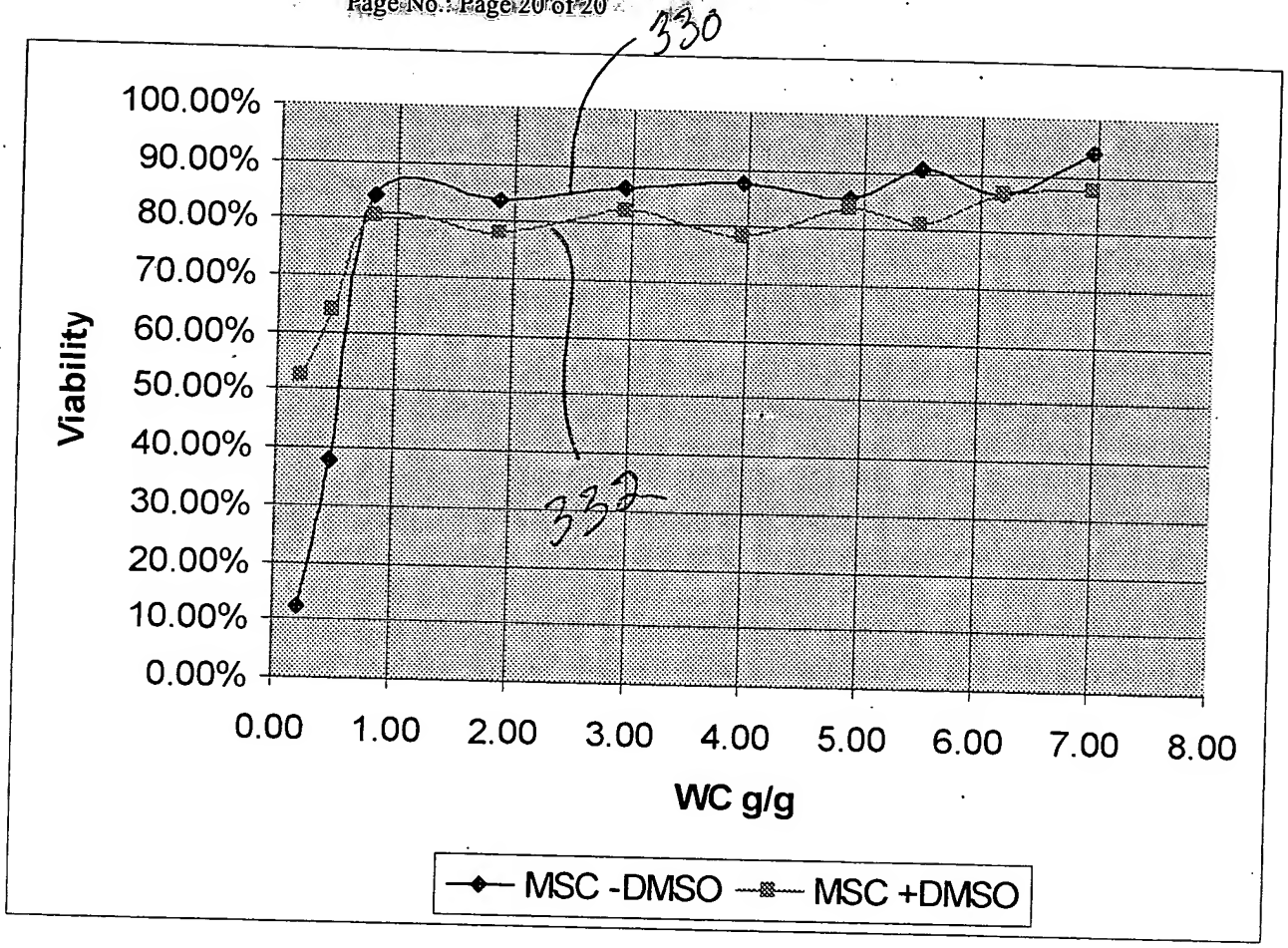

330
332
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Viability
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
WC g/g
MSC -DMSO
MSC +DMSO

Fig 33